

Stimulation of the Labellar Sugar Receptor of the Fleshfly by Mono- and Disaccharides

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ABSTRACT Responses of the labellar sugar receptor of the fleshfly, *Boettcherisca peregrina*, were studied over a wide range of concentrations of several sugars (sucrose, maltose, glucose, fructose, and mannose) in single solutions and in mixtures. The results suggest (a) that the receptor sites are not completely differentiated for glucose and for fructose combination, (b) that the receptor site is composed of two subunits. Such suggestions are based on the classical model, where the response is proportional to the number of the sites, two subunits of each site being simultaneously occupied with one molecule of disaccharides or two molecules of monosaccharides. It is shown, however, that an allosteric model gives a somewhat better interpretation of the experimental results.

INTRODUCTION

Among the taste receptors, the sugar receptor has the unique property that it is stimulated by uncharged molecules. This is quite remarkable, since almost all types of structures on the cell membrane (muscle end plates, neuron synapses, etc., as well as salt taste receptors) are stimulated only by charged molecules or ions. How can uncharged molecules produce electrical changes in the receptor membrane? This is one of the most interesting problems in membrane physiology. One approach to this problem is to study the relative stimulating effectiveness of different sugars and to evaluate the relation between the molecular structure of the sugar and its stimulating effectiveness. The final goal of this approach would be to discover the molecular structure of the site on the receptor membrane with which sugar molecules combine. In fact, much work thus oriented has been done in both vertebrates and invertebrates. These studies have been based on two groups of experiments. One estimates the threshold concentration of sugars for a definite behavioral response, including psychophysical experiments in man. Accordingly, the

sugars are tested at their very dilute concentrations. The other compares magnitudes of neural response of the receptor to a fixed concentration of different sugars. However, the order of effectiveness is not necessarily the same at different concentrations. Therefore, the effectiveness must be compared by recording the response of single receptors to sugars over a wide range of concentrations. That is, we have to investigate the response magnitude of the receptor vs. the concentration of each sugar. Such a study will give us another aspect of information; i.e., the mode of complex formation between the receptor site and the sugar molecules.

The labellar sugar receptor of many species of flies is the best material for quantitative studies. First, we can easily record its sensory activities from the sidewall of the labellar chemosensory hair (Morita, 1959). Second, it has been ascertained in this receptor that the receptor potential can be considered proportional to the impulse frequency (Morita and Yamashita, 1966). This implies that we can quantitatively, though not in their absolute values, discuss the displacement of the receptor membrane potential and the receptor membrane current by measuring the impulse frequency, since they are also proportional to the recorded receptor potential. Third, there is a phase which can be regarded as stationary in the sensory adaptation curve of the impulse (in the blowfly, Steinhardt, Morita, and Hodgson, 1966). We can deal with the response magnitude in this phase in the same way as with the rate of enzyme reaction, where a steady state is assumed in the process of formation of the enzyme-substrate complex. Last, but not least, we can obtain quantitatively reproducible responses in this receptor if the stimulus duration is kept below 0.5 sec (Steinhardt et al., 1966). This method of short stimulation was introduced by Evans and Mellon, and was applied successfully by them to the labellar water receptor of the blowfly (1962 *a*) and to the salt receptor (1962 *b*).

Morita, Hidaka, and Shiraishi (1966) showed that the results obtained in the sugar receptor of the fleshfly could be explained by assuming that the response magnitude is proportional to the number of the receptor sites, each of which is occupied by one molecule of sucrose. Such an assumption is the basis of Beidler's taste theory (Beidler, 1954), and was found to hold for the salt receptor of the rat (Beidler, 1954) and the labellar salt receptor (Evans and Mellon, 1962 *b*; but see Gillary, 1966). As to the receptor site, Evans (1963) has claimed that there are at least two different types in a single sugar receptor of the blowfly, one being the glucose-combining site and the other the fructose-combining one.

In the present work we have tried to clarify the properties of the receptor site of the labellar sugar receptor of the fleshfly, investigating the responses to solutions of sugars and mixtures of different sugars over wide ranges of concentration.

MATERIAL AND METHODS

The fleshfly, *Boettcherisca peregrina*, was used throughout this work. The larvae were raised on minced horse meat, and the imagos were raised in the same way, but fed also with 5% sucrose solution. Imagos between 3 and 6 days old were used in the experiments.

The recording and stimulation systems were almost the same as described elsewhere (Morita, 1959). An isolated proboscis was mounted on a piece of platinum wire which was inserted into the proboscis through the cut end and served as an indifferent electrode. A long hair on the marginal zone of the labellum was selected, and the sidewall of the hair was cracked with a microneedle about 50 μ from the tip by supporting the hair with the tip (about 15 μ in diameter) of a capillary electrode on the opposite side. Then, the cracking needle was replaced with a second capillary with a tip diameter of about 30 μ , its tip having been previously dipped briefly into the same electrolyte solution as that in the capillary electrode. When the tip of the capillary electrode was brought into contact with the surface of the solution in the second capillary, the solution began to move from the electrode to the second capillary. Thus, the solution near the surface was renewed continuously and condensation by evaporation at the electrode tip was prevented. Sensory activity was recorded from the cracked part of the hair which was kept in contact with this continuously renewed surface, and the receptor responded at least for 2 hr in a quantitatively reproducible manner unless the receptor was injured during the cracking procedure. The electrolyte solution used for the capillary electrode was Waterhouse's saline (Buck, 1953).

Stimuli contained in a third capillary whose tip diameter was 50–100 μ were applied to the receptor at the hair tip. Movement of this capillary was controlled by a small electromagnet, which was supplied with electric current by an electronic stimulator. The duration of stimulation never exceeded 0.5 sec. Intervals between stimuli were adjusted with various stimulus strengths. For example, in sucrose stimulation, the intervals were 1.5, 3, and 5 min after stimulation by solutions below 0.1, 0.1–0.2, and above 0.2 M, respectively. All experiments were done at ambient temperatures of $25^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$, and at relative humidities of 60–70%. The sensory impulses picked up from the cracked part on the sidewall were fed into an oscilloscope through an amplifier with grid leak of 10^{11} ohm and of low grid current (below 10^{-12} amp) in its head stage. They were then photographed on running oscillographic paper, and the impulses were counted for 0.15–0.3 sec from 0.15 sec after the beginning of the stimulus, as a measure of the magnitude of the stationary response. In the present paper this value will be referred to as the magnitude of response.

All sugars used were of special grade of Wako Chemical Industries, Ltd., Japan (D-form for monosaccharides), except for D-fructose, which was made by the British Drug Houses, Ltd., England. The specification attached to the fructose sample showed that the specific rotation $[\alpha]_{\text{D}}^{20}$ was -89 to -92 and that contamination from D-glucose was less than 1%.

The sugars were dissolved in distilled and deionized water for experimental use. Unless otherwise stated, concentrations of sugars are expressed in molarity. This was

for convenience in preparing solutions over a wide range of concentrations. We plotted concentrations principally on logarithmic scales, so that the difference between molarity and molality (and thermodynamic activity, too) is relatively small.

RESULTS

Responses to Single Sugars

COMPARISON OF SUCROSE, GLUCOSE, AND FRUCTOSE Fig. 1 shows one of the examples in which the responses of a single sugar receptor to sucrose,

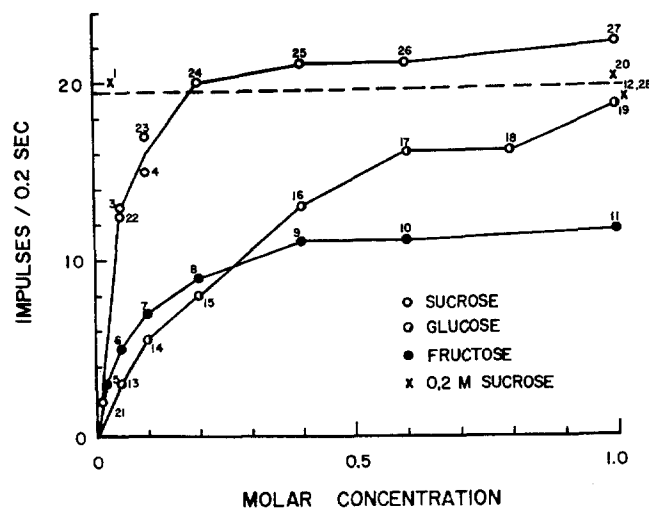


FIGURE 1. Comparison of response-concentration relation in stimulations by sucrose, glucose, and fructose. Number attached to each symbol indicates the order of stimulation.

glucose, and fructose were studied over a range of concentration between 0.01 and 1.0 M. The numbers attached to the circles represent the order of stimulation. As the numbers show, stimulations were given in ascending order as to the concentration of the sugar used. The receptor was stimulated by 0.2 M sucrose at times to check the reproducibility of the magnitude of the response. Such procedures were routine for other experiments in the present work.

The results of Fig. 1 show that the order of stimulating effectiveness was sucrose > fructose > glucose below 0.3 M, but that it changed to sucrose > glucose > fructose above 0.3 M. Sucrose was the most effective at all concentrations and in all preparations. Between fructose and glucose the concentration at which the order changed varied with the preparation, ranging from 0.1 to 0.4 M, but the reversal was observed in all preparations.

Table I shows a comparison of the responses to sucrose, glucose, and fruc-

tose at their maximum values experimentally obtained at high concentrations. These values would not be the same as the maximum response each sugar could produce. The values were obtained, however, from a series of experiments, in which the concentration range extended above 1.0 M and the response-concentration curve showed a practically horizontal line at high concentrations. Therefore, as rough estimations of the maximum response, relative to that to sucrose, we may take averages of 0.71 and 0.46 in responses to glucose and fructose, respectively.

Hassett, Dethier, and Gans (1950) studied the relative sensitivity of the blowfly to the three sugars, comparing individual ascending tarsal thresholds to the sugars for behavioral response. Their results indicated that fructose

TABLE I
COMPARISON OF MAXIMAL RESPONSES TO
SUCROSE, GLUCOSE, AND FRUCTOSE

Preparation	Sucrose	Glucose	Fructose
275	1.0	0.26	0.33
274	1.0	0.73	0.4
273	1.0		0.52
218	1.0	0.69	0.56
216	1.0		0.45
215	1.0		0.40
214	1.0		0.53
213	1.0	0.78	
208	1.0	0.73	
Average	1.0	0.71	0.46

was the most effective (twice as effective as sucrose), sucrose next, and glucose least. They used different experimental methods, species of fly, and receptor locations, but their results correspond to ours at low stimulus concentrations. Discrepancy between the two works is obvious when the relative sensitivities to sucrose and fructose are under consideration. We considered impurity in our fructose as one of the causes for the discrepancy, and used D-fructose (extra pure for injection) made by E. Merck (Germany) and found no difference in the results for the two fructose samples. However, there is also the possibility of the same sort of impurity existing in the fructose made by E. Merck.

TENTATIVE MODEL For the case in which the response magnitude is proportional to the number of sites, each of which is occupied by one stimulus molecule, we can use an equation similar to the Michaelis-Menten equation which describes enzyme reactions. Lineweaver and Burk (1934) modified this equation and introduced two types of plots, giving straight-line relation-

ships. Fig. 2 corresponds to the second type of the Lineweaver-Burk plot, and is the same as that which Beidler (1954) first applied to the chemoreceptor. A straight-line relationship was obtained for sucrose, as observed by Morita et al. (1966), but not for glucose. This means that the response to glucose is not proportional to the number of 1:1 complexes as formed between the glucose molecule and the receptor site. We, therefore, examined a model of a 2:1

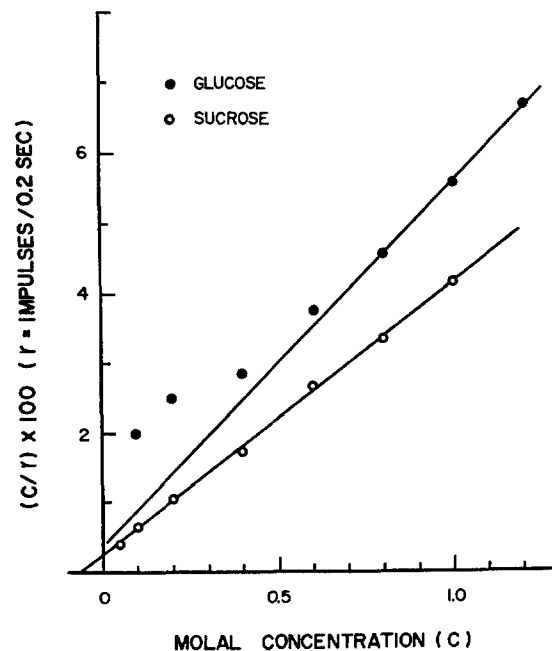


FIGURE 2. Beidler's (or Lineweaver-Burk) plot in stimulations by glucose and sucrose. Deviation from a straight line in stimulation by glucose shows that the response cannot be described as Beidler's (or the Michaelis-Menten) type.

complex formation between stimulus molecules and the receptor site as the simplest after that of a 1:1 complex.

When the 2:1 complex is formed, the reaction may generally be divided into two steps as



and



where A , S , AS , and A_2S represent a molecule of stimulus substance, the receptor site, and the 1:1 and 2:1 complexes, respectively. Allowing a , n_1 , and n_2 to be the concentration or number of A , AS , and A_2S , respectively, and s the

sum of the numbers of S , AS , and A_2S , we may assume the existence of constants, K_1 and K_2 (not necessarily equal to dissociation constants), in a steady state, as

$$K_1 = a(s - n_1 - n_2)/n_1, \quad (3)$$

and

$$K_2 = an_1/n_2. \quad (4)$$

If the magnitude of response, r , is proportional to the number of the complexes, A_2S , as formed, the following equation is obtained:

$$r/r_m = 1/(1 + K_2/a + K_1K_2/a^2), \quad (5)$$

where r_m is the maximal response resulting from all the sites being occupied by two molecules of the stimulant, so that the value of r/r_m may be called the relative response. Introducing the "relative concentration," $c = a/K_1$, and a constant, $\alpha = K_2/K_1$, we can rewrite equation (5) as

$$r/r_m = 1/(1 + \alpha/c + \alpha/c^2). \quad (6)$$

This equation shows that the curve representing the value of r/r_m plotted against c is determined by the value of α ; that is, the curves representing equation (5) with the same value of K_2/K_1 are the same in shape when plotted against a on a logarithmic scale, even if the values of K_1 are different. Thus, the test of the experimental results with equation (5) is facilitated to a great extent.

Disaccharides Before going into the results with monosaccharides, let us describe the results with sucrose, when the 1:1 complex model was considered to hold as shown by Fig. 2. In this case, the response is proportional to n_1 , and n_2 is zero because $K_2 = \infty$. Accordingly, the relative response is expressed as

$$r/r_m = 1/(1 + K_1/a). \quad (7)$$

The theoretical curve calculated from equation (7) is compared in Fig. 3 with experimental values obtained in a single sugar receptor. In this and the two following figures, the experimental values were reduced by 2–5% to get the best fit at high sugar concentrations.

As seen in Fig. 3, the theoretical curve fits the experimental values fairly well over the range 0.001 to 1.5 M sucrose. Slight deviation from the curve is seen at concentrations from 0.003 to 0.03 M, and may be significant as shown also in Fig. 4 by plots of results with six preparations. The value of the

constant, K_1 , varied with preparations, but, if the response is expressed by equation (7), all experimental values should be represented by a single curve plotted against the relative concentration, even with different K_1 values in different preparations. As Fig. 4 shows, the results of six different preparations (filled circles) can be thought of as expressed by a single theoretical curve,

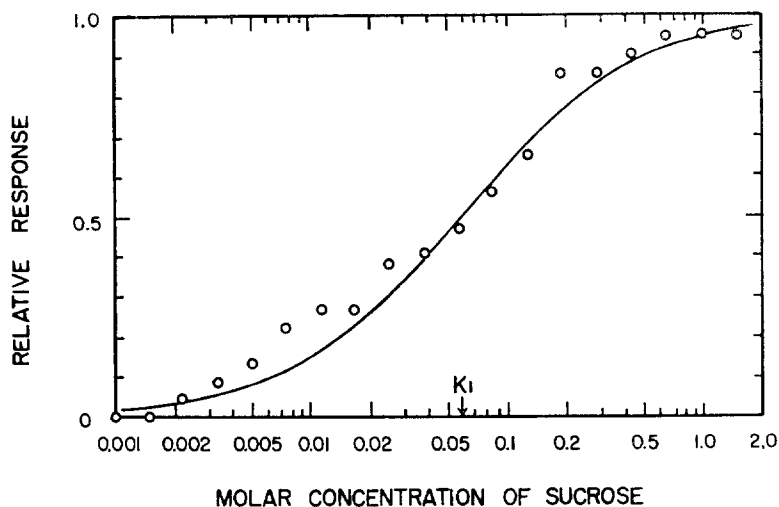


FIGURE 3. Responses relative to the maximum vs. concentration of sucrose plotted on a logarithmic scale (in a single receptor). Open circles represent experimental values and the continuous line represents the theoretical curve calculated by the equation (response of Beidler's or Michaelis-Menten type):

$$r/r_m = 1/(1 + K_1/C)$$

where r is the magnitude of response; r_m , the maximum response when all the sites are occupied each by one molecule of sucrose; K_1 , a constant corresponding to the dissociation constant of a 1:1 complex between the site and sucrose molecule; C , molar concentration of sucrose. The maximum responses experimentally obtained are estimated as 95% of the true maximum which the receptor could reach, so that the best fit is obtained at high concentrations of sucrose.

but, here also, deviation from the curve is obvious over the range from 0.05 to 0.5 in the relative concentration.

Fig. 5 shows the results with maltose chosen as another disaccharide and treated in the same way as in Fig. 4. Fig. 5 includes the results with five different preparations. The results with the individual preparations were found to be expressed by the theoretical curves calculated from equation (7), and there was not the deviation seen with sucrose.

In the results shown in Figs. 4 and 5, the value of K_1 for sucrose was 0.06 M, ranging from 0.05 to 0.14 M (with six preparations); that for maltose was 0.1 M, ranging from 0.05 to 0.15 M (with 5 preparations) (see Table II).

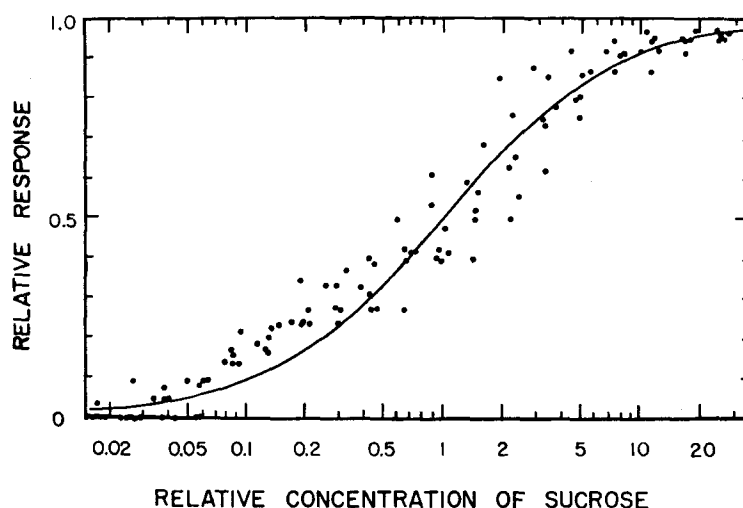


FIGURE 4. The same type of test as in Fig. 3, but with experimental values (filled circles) in six different receptors. Relative concentration is C/K_1 , where C is molar concentration. Continuous line represents the theoretical curve calculated with the same equation used in Fig. 3. Experimental values were reduced as in Fig. 3.

Monosaccharides Figs. 6 and 7 show tests of the results with fructose and glucose, respectively, by equation (6). As seen in these figures, the experimental values (filled circles) are in good agreement with the theoretical curves (continuous curves) of the 2:1 complex model. Fig. 6 includes the results with nine different preparations, and the value of α ($= K_2/K_1$) for

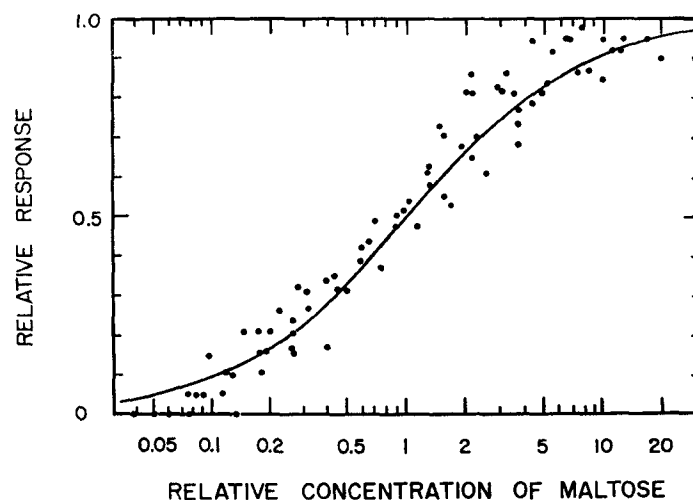


FIGURE 5. The same as in Fig. 4, but in stimulation by maltose, with five different receptors.

the theoretical curve was 4. Fitting the theoretical curve with the experimental values obtained from one preparation, the value of K_1 for this preparation was determined by reading the concentration corresponding to unity in the relative concentration. The values of K_1 thus obtained for individual preparations ranged from 0.014 to 0.025 M and were averaged as 0.015 M. Accordingly, the average of K_2 values was 0.06 M ($K_2/K_1 = \alpha = 4$). Similarly, for glucose (Fig. 7) tested on 10 different preparations, α was unity, and the averaged value of K_1 was 0.1 M, ranging from 0.08 to 0.12 M.

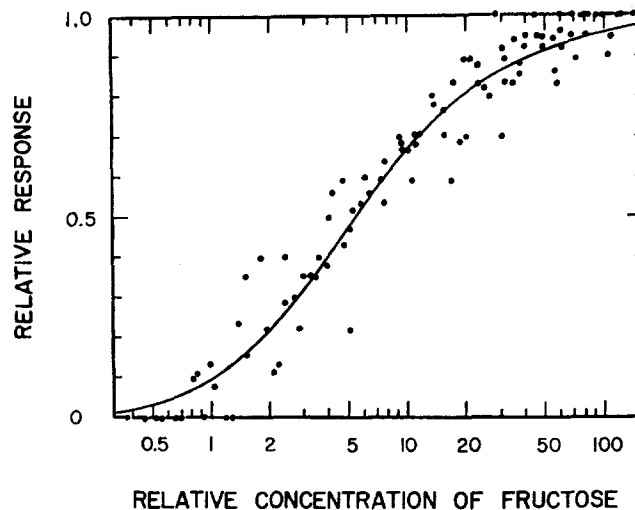


FIGURE 6. Comparison of experimental results (filled circles) in nine receptors stimulated by fructose with the theoretical curve (continuous line) calculated from the equation:

$$r/r_m = 1/(1 + \alpha/c + \alpha/c^2),$$

where $c = C/K_1$ (relative concentration), $\alpha (= K_2/K_1) = 4.0$ (see equations 3 and 4 in the text as to K_1 and K_2), the others the same as in Fig. 3.

There was a distinct difference between the results with mono- and disaccharides. While the results with disaccharides with the individual preparations were all expressed by the same theoretical curve plotted against the relative concentration, those with monosaccharides were not. This is because the value of α varied with preparations. Nevertheless, the results with different preparations were on the whole expressed by a single theoretical curve for each sugar as shown in Figs. 6 and 7. This means that there was a mean in the value of α for each monosaccharide, and this value varied randomly about the mean with different preparations.

In Table II are summarized the values of K_1 and K_2 estimated as above.

The table shows that fructose is the most effective in the sense that it has the highest affinity for the receptor site.

Interactions between Different Sugars

It has been shown above that stimulations by disaccharides and by monosaccharides are explained by the 1:1 and 2:1 complex models, respectively.

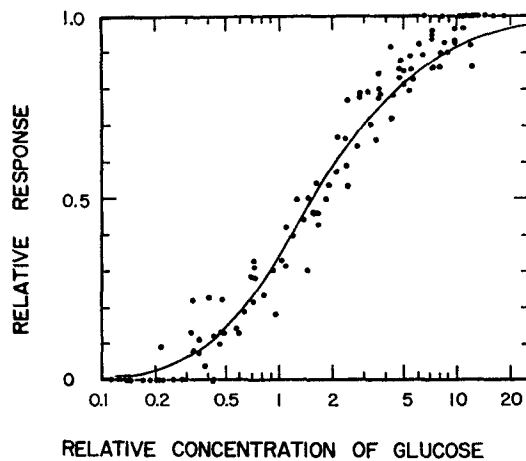


FIGURE 7. The same as in Fig. 6, but with 10 receptors stimulated by glucose. The value of α used for calculation of the theoretical curve is 1.0.

TABLE II
APPARENT K_1 , K_2 VALUES FOR THE SUGARS
TO COMPLEX WITH THE RECEPTOR SITE

Sugars	K_1	Range of K_1	K_2
	<i>M</i>		<i>M</i>
Sucrose	0.06	0.05–0.14	
Maltose	0.1	0.05–0.15	
Fructose	0.015	0.014–0.025	0.06
Glucose	0.1	0.08–0.12	0.1

For the meaning of K_1 and K_2 , see equations (3) and (4) in the text.

The structure or nature of the receptor site will be described below while the interaction between different sugars stimulating the same receptor is also investigated.

“GLUCOSE AND FRUCTOSE COMBINING SITES” Evans (1963) has postulated that there are at least two types of combining sites on the membrane of one sugar receptor, one for glucose and the other for fructose. He has also claimed that sucrose acts predominantly at the “fructose site” (Evans, 1961). If these sites are strictly specific for each substrate (i.e. sucrose combines only with the fructose site), there would be little interaction between sucrose and glucose

when the two sugars are given in the same solution. One of the experimental results is shown in Fig. 8, where the responses to plain sucrose solutions are compared with the responses to mixtures of 1 M glucose and various concentrations of sucrose. Concentrations of sucrose in the plain and mixed solutions are plotted on the X axis. The results show that the response to the mixture of 1 M glucose and one of the various concentrations of sucrose could be regarded as slightly additive only at a low concentration of sucrose. The response to the same concentration of sucrose was higher in the plain solution than in the

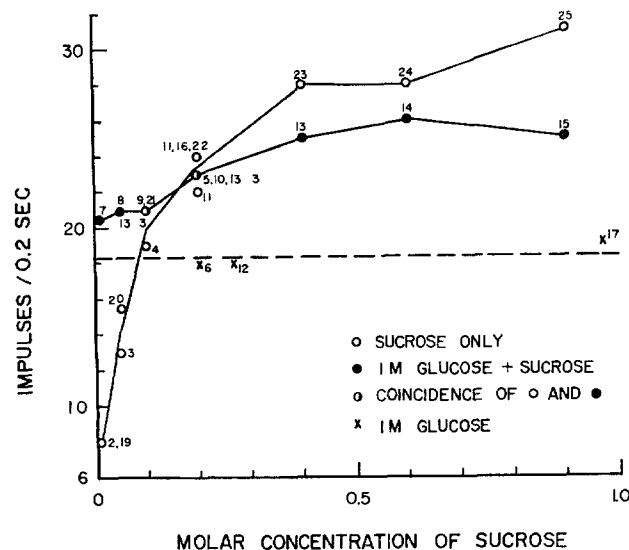


FIGURE 8. Effects of 1 M glucose on stimulation of a single receptor by sucrose. Numbers attached to symbols represent the order of stimulations (the same in the following figures). The broken line shows the average of responses to 1 M glucose. Values on X axis represent concentrations of sucrose both in plain solutions and in mixtures with 1 M glucose.

mixture at high concentrations of sucrose. Therefore, we cannot conclude that the sucrose molecule combines only with one type of site, quite independently of the "glucose site." On the contrary, we will have to assume fairly strong competition between sucrose and glucose for the same receptor site.

Almost the same extent of interaction was observed between sucrose and fructose (Fig. 9). Compared with the inhibition by glucose shown in Fig. 8, that by fructose was no stronger. These results show that sucrose molecules combine with the glucose site as well as with the fructose site, if there is any differentiation among the receptor sites.

Interaction between glucose and fructose should give us information about the differentiation between the glucose and fructose sites. The results of the

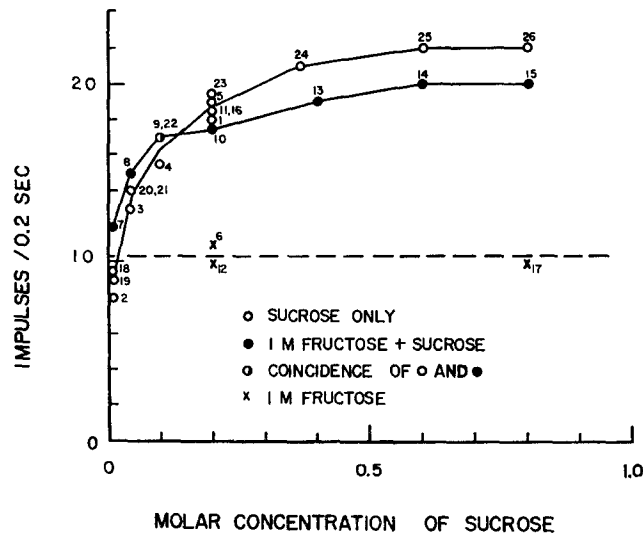


FIGURE 9. The same as in Fig. 8, but with interaction between fructose and sucrose.

experiment as they affect this problem are shown by Fig. 10. The responses to the mixtures of 0.05 M glucose and various concentrations of fructose were higher, apparently by an amount of the response to 0.05 M glucose, than those to plain fructose solutions over the entire range of fructose concentrations tested. Accordingly, the glucose site might be assumed to be differentiated from the fructose site. However, the lack of significant difference between the response to the mixtures of 1 M glucose with fructose (half-filled circles) and

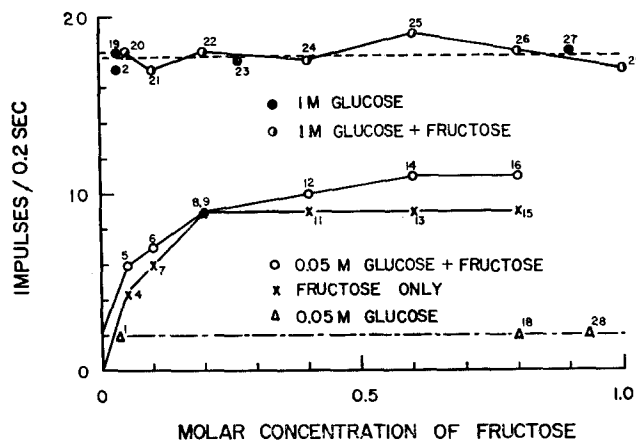


FIGURE 10. Interaction between glucose and fructose. Concentration of glucose in mixtures was fixed at 1.0 or 0.05 M. Averages of responses to 1.0 and 0.05 M glucose are represented by broken and dot and dash lines, respectively.

that to plain 1 M glucose (filled circles) suggests that glucose molecules can occupy the fructose site fairly accurately, competing with fructose molecules. This means that the differentiation is poor, if it exists at all. The apparent additivity mentioned with the mixture of 0.05 M glucose and fructose may be explained by assuming that the complex type, such as the fructose-glucose-receptor site, can be formed (see Fructose effects).

MANNOSE EFFECTS Mannose has been known as a unique monosaccharide. In spite of a very weak stimulating effect, it is a strong competitive inhibitor for fructose stimulation according to the behavioral study on the blowfly by Dethier, Evans, and Rhoades (1956). The results shown in Fig. 11

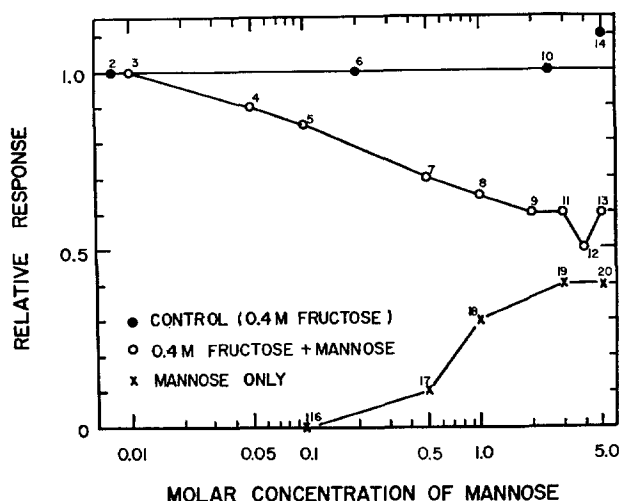


FIGURE 11. Effects of various concentrations of mannose on stimulation by a fixed concentration of 0.4 M fructose, where response was taken as unity.

verify their conclusion. Here, again, the response to plain mannose solutions cannot be explained by the 1:1 complex model, since, in that case, the response-intensity curve should cover a concentration range of 1 to 10^3 for zero to the maximum response (see Figs. 3–5), whereas the curve in the mannose response covers a concentration range of less than 1 to 10^2 (see also Figs. 12 and 13). Furthermore, mannose has a definite effect on the response to fructose at concentrations at which mannose does not have any stimulating effect by itself. This suggests that a mannose molecule, at these concentrations, occupies one of the two units for fructose molecules in a receptor site and blocks the response.

The results of the same type of experiments for glucose and sucrose are shown in Figs. 12 and 13. At concentrations (around 0.05 M) of no stimulating effect by itself, mannose apparently had a synergistic effect on the responses to glucose and sucrose. Also in the results shown in Figs. 12 and 13 an inhibition

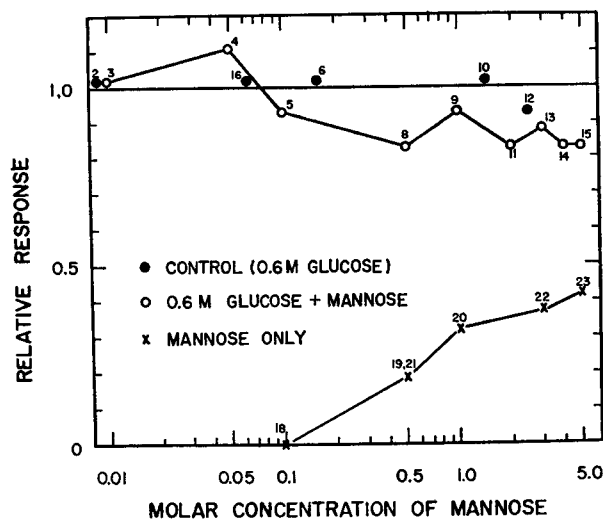


FIGURE 12. Effects of various concentrations of mannose on stimulation by a fixed concentration of 0.6 M glucose, where response was taken as unity.

by mannose is observed, but it was weak compared with the effect on fructose and occurred at concentrations at which mannose could stimulate by itself.

FRUCTOSE EFFECTS When the results presented above are considered, we might imagine the simplest picture of sugar stimulation as follows. The receptor site with which one sucrose molecule combines is comprised of two subunits. For excitation the receptor site has to be simultaneously occupied

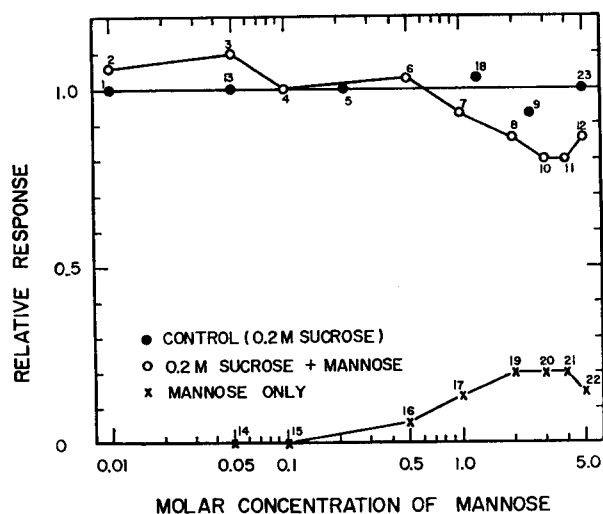


FIGURE 13. Effects of various concentrations of mannose on stimulation by a fixed concentration of 0.2 M sucrose, where response was taken as unity.

at the two subunits. Disaccharides could fill the two units with one molecule, but monosaccharides would have to fill them with two molecules.

A problem here is the behavior of fructose. As shown in Table II fructose is considered to have the highest affinity (even if the value of K_2 is taken into account) among the sugars tested. Nevertheless, its competitive effect on stimulation by other sugars was very weak (see Figs. 9 and 10), and its stimulating effect was deeply depressed by low concentrations of mannose (Fig. 11). Another example of such a property of fructose is shown by experiments in which the receptor was stimulated by mixtures of 0.1 M sucrose and various

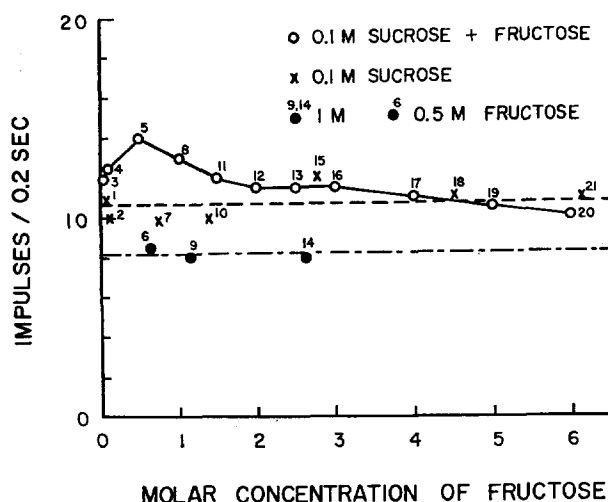


FIGURE 14. Effects of various concentrations of fructose on stimulation by a fixed concentration of 0.1 M sucrose. Averages of responses to 0.1 M sucrose and the maximal response to fructose experimentally obtained are represented by broken and dot and dash lines, respectively.

concentrations of fructose, up to 6 M (Fig. 14). From the values of K_1 (shown in Table II) and the concentration ratio, sucrose molecules should occupy less than $1/240$ of the total of the receptor sites in the mixture of 0.1 M sucrose and 6 M fructose, and the response to the mixture should be almost the same as the maximum response to fructose, provided that a sucrose molecule never shares one of the sites with another molecule. The response was not reduced significantly, however, compared with that to single solutions of 0.1 M sucrose. One explanation for such a result is simply to assume that one receptor site can be shared by each of the sucrose and fructose molecules. If such a heterogeneous complex is more effective in excitation than the 2:1 complex of fructose and the receptor site, the inhibitory effect of fructose on stimulation by other sugars should be relatively slight.

The above-mentioned assumption predicts that the complex (fructose-

glucose-receptor site) is formed when the sugar receptor is stimulated by a mixture of fructose and glucose, and can be detected as a synergism at low concentrations. Dethier et al. (1956) reported that this was the case, but probably because of the variability of the K_1 value with different preparations and other factors, the prediction was fulfilled by only one preparation out of several (Fig. 15). In this preparation, the responses to the mixtures of 0.02 M fructose with glucose (notice the results below 0.04 M glucose) are shown to be higher than those to the pure solution of 0.02 M fructose, though responses to glucose below 0.04 M were zero.

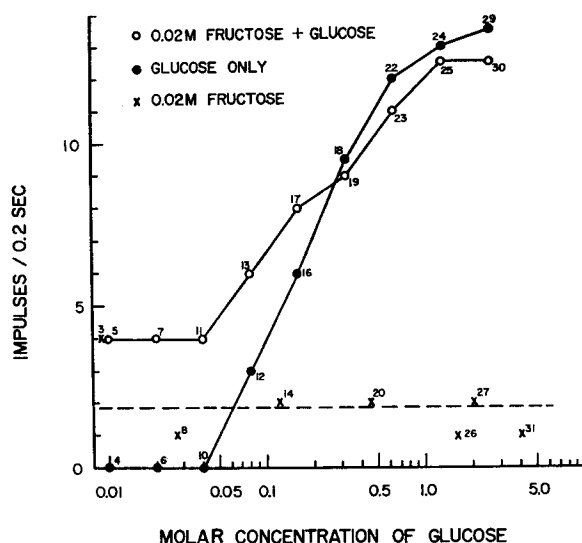


FIGURE 15. Effects of various concentrations of glucose on stimulation by a fixed concentration of 0.02 M fructose. Average of response to 0.02 M fructose is represented by broken line.

DISCUSSION

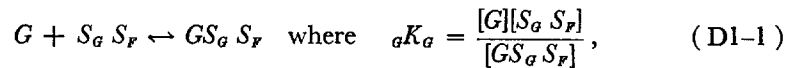
Multimolecular Complex Model Formation of a multimolecular complex between stimulating molecules and the receptor site described here is not the first example to be shown in chemoreceptors. Quite recently, Tateda and Hidaka (1966) studied the receptor for sweet substances in the rat, and have suggested that more than four molecules of glycine can combine with the single receptor site.

In the present work, we need not have assumed any model of a complex combining more than two molecules of stimulant. In describing the results, we have assumed only one type of receptor site, which is divided into two subunits: when the two subunits are simultaneously filled with stimulating molecules, excitation results. From this picture of the sugar receptor, it also

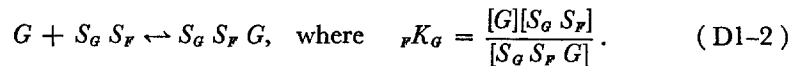
follows that the single receptor site could combine with two molecules of disaccharides. This point of view might assist in interpreting the slight deviation from the theoretical curve shown in the results with sucrose stimulation.

Differentiation of the Receptor Site The results of strong interaction between glucose and fructose suggest that no definite groups of receptor sites are differentiated for combining only with two molecules of glucose or only with two of fructose. The same results, however, do not exclude the possibility that the two subunits of the receptor site differentiate to combine with glucose and fructose, respectively. From the affinity, which is measured by the reciprocals of K_1 and K_2 values listed in Table II, it is also unlikely that there is any complete differentiation between the subunits, since the K_2 value for fructose is less than the K_1 value for glucose. (If there is any differentiation for glucose and for fructose, the first step of the reaction in stimulation by glucose should occur mainly at the subunit specific for glucose, and the second step mainly at the one specific for fructose.) However, some degree of differentiation is suggested by the difference in the effects of mannose on stimulation by fructose and by glucose or sucrose.

If we assume temporarily that the subunits are differentiated, and, therefore, denote them by S_G and S_F , respectively (the receptor site, S , accordingly, being expressed as $S_G S_F$) and the glucose molecule by G , equation (1) is divided into



and



Each symbol in equation (D1) corresponds to each one in equations (1) and (3) as

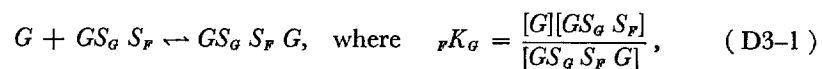
$$G = A, \quad S_G S_F = S, \quad GS_G S_F \quad \text{and} \quad S_G S_F G = AS,$$

$$[G] = a, \quad [S_G S_F] = s - (n_1 + n_2), \quad \text{and} \quad [GS_G S_F] + [S_G S_F G] = n_1.$$

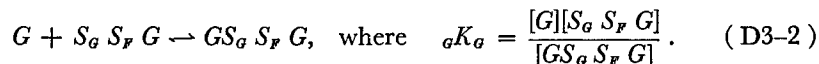
Comparing equation (D1) with equations (1) and (3), we obtain

$$\frac{1}{{}_G K_G} + \frac{1}{{}_F K_G} = \frac{1}{K_1}. \quad (\text{for glucose}) \quad (\text{D2})$$

The second step of reaction is assumed to proceed as



and



Then, comparing equation (D3) with equations (2) and (4), we get

$${}_G K_G + {}_F K_G = K_2. \quad (\text{for glucose}) \quad (\text{D4})$$

The value of $\alpha (= K_2/K_1)$ for glucose can be obtained from equations (D2) and (D4) as

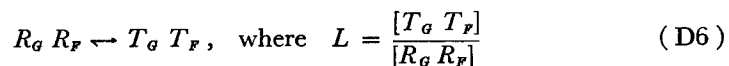
$$\alpha = K_2/K_1 = ({}_G K_G + {}_F K_G)^2 / ({}_G K_G \cdot {}_F K_G). \quad (\text{D5})$$

It can easily be shown from equation (D5) that the value of α is minimum and is 4 when ${}_G K_G = {}_F K_G$. Therefore, any value of α below 4 indicates that the assumption made in equation (D3) is wrong, and that a "stabilizing interaction" exists between the subunits.

The analyses of the results of glucose and fructose stimulation are summarized in Table II, and the value of $\alpha = K_2/K_1$ is unity for glucose and 4 for fructose. As far as we can assume that there are two subunits in a single receptor site, we have to conclude that there is a stabilizing interaction between the subunits making a complex with molecules of monosaccharide.

Allosteric Model Such an interaction as the one mentioned above has been claimed as strong evidence for allosteric transition in proteins (Wyman, 1963). Our receptor site is considered in many respects to be composed of allosteric macromolecules. First, specificity for certain sugars may be attributed only to macromolecular structure. Second, noncharge molecules such as those of sugars may induce electrical changes in the receptor membrane only through structural changes in the receptor site, and these changes should be closely related to, or synonymous with, allosteric transitions. Third, the existence of subunits has been emphasized in the present paper, and an interaction between the subunits has been suggested. It is, therefore, justifiable to examine the present results from the viewpoint of allosteric transitions in the receptor site.

Monod, Wyman, and Changeux (1965) have proposed a model for allosteric transitions. According to them, let us assume two states of the receptor site as



and R_G , R_F , T_G , and T_F represent two different subunits in the R and T state, respectively. The symbol, L , denotes an equilibrium constant for the

transition. The dissociation constants between the subunits and ligands (fructose and glucose) are defined as

$$\begin{aligned} {}_gK_g &= \frac{[G][R_g]}{[R_g G]}, & {}_fK_g &= \frac{[G][R_f]}{[R_f G]}, & {}_gK'_g &= \frac{[G][T_g]}{[T_g G]}, & {}_fK'_g &= \frac{[G][T_f]}{[T_f G]}, \\ {}_fK_f &= \frac{[F][R_f]}{[R_f F]}, & {}_gK_f &= \frac{[F][R_g]}{[R_g F]}, & {}_fK'_f &= \frac{[F][T_f]}{[T_f F]}, & {}_gK'_f &= \frac{[F][T_g]}{[T_g F]}. \end{aligned}$$

For convenience of derivation of the following equations, ratios between the constants are defined as

$$\begin{aligned} c_g &= {}_gK_g / {}_gK'_g, & b_g &= {}_gK_g / {}_fK_g, & b'_g &= {}_gK'_g / {}_fK'_g, \\ c_f &= {}_fK_f / {}_fK'_f, & b_f &= {}_fK_f / {}_gK_f, & b'_f &= {}_fK'_f / {}_gK'_f. \end{aligned}$$

The relative concentrations of glucose and fructose are denoted by $\alpha_g (= [G] / {}_gK_g)$ and $\alpha_f (= [F] / {}_fK_f)$, respectively. The numbers of all forms of the site in the R state, ΣR , and that in the T state, ΣT , are

$$\begin{aligned} \Sigma R &= [R_g R_f] + [G R_g R_f] + [R_g R_f G] + [G R_g R_f G] + [G R_g R_f F] \\ &\quad + [F R_g R_f G] + [R_g R_f F] + [F R_g R_f] + [F R_f R_g F] \\ &= [R_g R_f] \{ 1 + (1 + b_g) \alpha_g + b_g \alpha_g^2 + (1 + b_f b_g) \alpha_g \alpha_f \\ &\quad + (1 + b_f) \alpha_f + b_f \alpha_f^2 \} \end{aligned} \quad (D7-1)$$

$$\begin{aligned} \Sigma T &= [T_g T_f] + \dots \dots \dots (\text{the same types of complex as in } \Sigma R) \\ &= L [R_g R_f] \{ 1 + (1 + b'_g) c_g \alpha_g + b'_g c_g^2 \alpha_g^2 + (1 + b'_g b'_f) c_g c_f \alpha_g \alpha_f \\ &\quad + (1 + b'_f) c_f \alpha_f + b'_f c_f^2 \alpha_f^2 \}. \end{aligned} \quad (D7-2)$$

The function of state (fraction of the site in the R state), \bar{R} , is

$$\bar{R} = \frac{\Sigma R}{\Sigma R + \Sigma T}. \quad (D8)$$

The maximal value of the function of state, \bar{R}_∞ , when the values of α_f and α_g are infinitely large in an equimolar mixture of glucose and fructose, is written as

$$\bar{R}_\infty = \frac{1}{1 + \frac{c_g^2 L (\beta \gamma + b'_g) (\beta \gamma b'_f + 1)}{(\gamma + b_g) (\gamma b_f + 1)}}, \quad (D9)$$

where $\beta = c_f / c_g$ and $\gamma = \alpha_f / \alpha_g$. In the case of pure glucose or fructose, respectively, equation (D9) reduces to

$$\bar{R}_{\infty G} = \frac{1}{1 + c_G^2 L (b'_G/b_G)}, \quad (\text{D10-1})$$

$$\bar{R}_{\infty F} = \frac{1}{1 + c_F^2 L (b'_F/b_F)}. \quad (\text{D10-2})$$

If we assume that the response is proportional to \bar{R} , we can test this assumption with appropriate values for the constants.

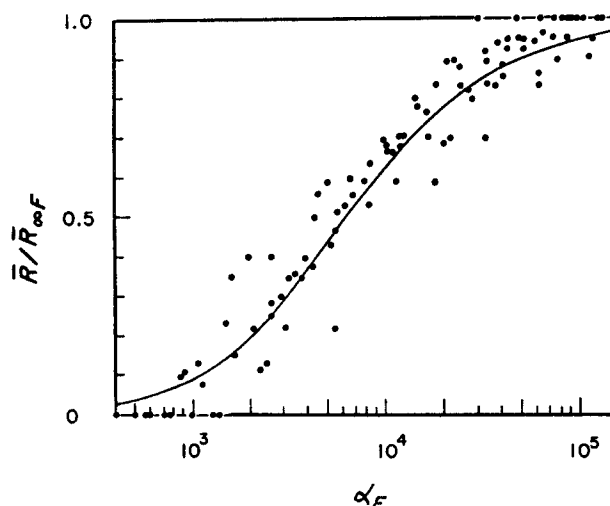


FIGURE 16. Test of the experimental results in stimulations by fructose used in Fig. 6 (filled circles) with allosteric model. The continuous curve was obtained from equation (D8) in the text. The values of the constants used:

$$L = 10^6, \quad c_F = 10^{-4}, \quad b_F = 0.1, \quad \text{and} \quad b'_F = 10.$$

Resultant value of $\bar{R}_{\infty F}$ is 0.5, and the estimated value of ${}_F K_F$ is 1.5×10^{-5} M.

The results of the test are shown in Figs. 16 and 17 for fructose and for glucose, respectively, and the experimental values are the ones used in Figs. 6 and 7. The constants used are

$$\begin{aligned} L = 10^6, \quad c_G = 10^{-4}, \quad b_G = 0.1, \quad b'_G = 1, \\ c_F = 10^{-4}, \quad b_F = 0.1, \quad b'_F = 10, \end{aligned} \quad (\text{D11})$$

so that the maximum responses for glucose ($\bar{R}_{\infty G}$) and for fructose ($\bar{R}_{\infty F}$) are calculated as 0.91 and 0.5, respectively; the values of ${}_G K_G$ and ${}_F K_F$ can also be estimated approximately as 4×10^{-5} and 1.5×10^{-5} M, respectively. In Fig. 18, the calculated values of \bar{R} ($\bar{R} = 1.0$ when all sites are in the R state) with the same values of constants used in Figs. 16 and 17 are plotted against molar concentration, so that it is easy to compare them with the experiments

shown by Fig. 1. The theoretical curves which were calculated for glucose (curve *A*) and for fructose (curve *B*) well represent the experimental data. Relative concentrations of 1 M glucose and fructose are approximated from the values of ${}_G K_G$ and ${}_F K_F$ (4×10^{-5} and 1.5×10^{-5} M, respectively) as $\alpha_G = 2.5 \times 10^4$ and $\alpha_F = 7 \times 10^4$. Therefore, with these values, the response to the mixture of 1.0 M glucose and 1.0 M fructose can be calculated by equation (D8), or as a close approximation by equation (D9). The resultant value is 0.77, and it is shown by the broken line at the upper part of

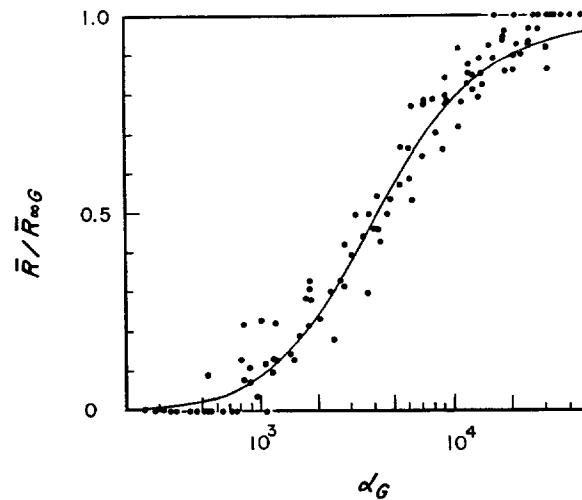


FIGURE 17. The same as in Fig. 16, but for stimulations by glucose. The experimental values (filled circles) are the same ones used in Fig. 7. The values of the constants used for calculation in equation (D8):

$$L = 10^6, \quad c_G = 10^{-4}, \quad b_G = 0.1, \quad \text{and} \quad b'_G = 1.$$

Resultant value of $\bar{R}_{\infty G}$ is 0.91, and the estimated value of ${}_G K_G$ is 4×10^{-5} M.

Fig. 18. This demonstrates that it is possible for the response to the mixture not to depart so much from the response to 1.0 M glucose, but to do so from the response to 1.0 M fructose. For comparison with the results in Fig. 10, the \bar{R} values for the mixtures of a dilute concentration of glucose and various concentrations of fructose ($\alpha_G = 10^3$, α_F is variable) are plotted as curve *C*. Compared with the results shown in Fig. 10 the result is somewhat too high at low fructose concentrations.

It will be noticed that the theoretical curves obtained from the allosteric and the classical complex models are almost the same. This is quite understandable since the quadratic terms of concentration (α_G^2 , α_F^2 and $\alpha_G \alpha_F$) are by far the largest in size in the numerator of equation (D8). In other words, in the allosteric model also the response is practically proportional to

the number of sites occupied by two molecules of ligand. Some important differences between the two models, however, exist in the assumptions on which the two are based. It has been shown that the maximal responses are different with different sugars. According to the allosteric model, such differences result mainly from differences in the ratios between the dissociation constants of ligands for the *R* state and for the *T* state of the receptor site. The classical complex model, however, interprets the same result as the difference in the proportionality constant between the response magnitude and the number of the 2:1 complex. It might be supposed, therefore, that the extent

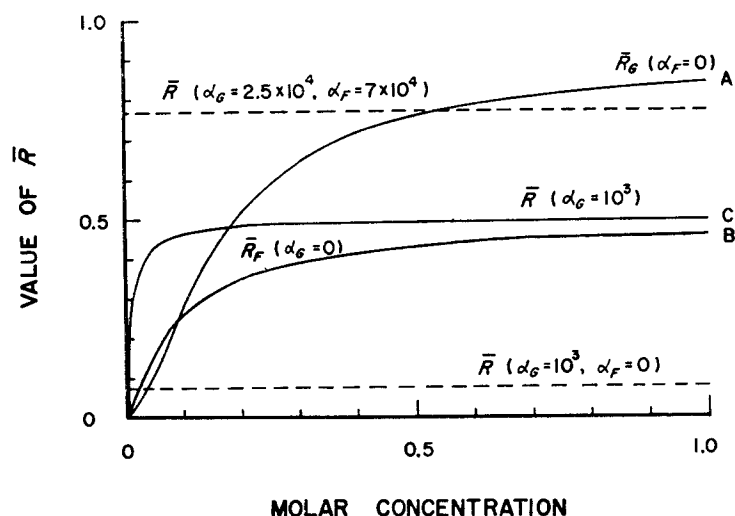


FIGURE 18. Calculations of fractions of the site in the *R* state, \bar{R} , for various concentrations of glucose (curve A), of fructose (curve B), both in plain solutions; and of fructose in mixtures with glucose whose relative concentration is 10^3 (curve C). Calculated values of \bar{R} for the mixture of 1 M glucose ($\alpha_G = 2.5 \times 10^4$) and 1 M fructose ($\alpha_F = 7 \times 10^4$) and for plain glucose at relative concentration of 10^3 are represented by the upper and lower broken lines, respectively. The values of the constants used are the same that were used in Figs. 16 and 17.

of permeability change in the receptor membrane could be different with different types of the complex.

If we assume conventionally that the K_1 values in Table II are the dissociation constants and compare them with the values of ${}_G K_G$ and ${}_F K_F$ estimated from the allosteric model, the free energy change for forming a complex with the ligands has to be more negative in the *R* state of the allosteric model than in the site of the classical complex model by 7–8 kcal/mole. This amounts to the free energy change of hydrolysis of so-called “high energy” phosphate compounds. In fact, in the allosteric model, combination of the ligands with the subunit in the *R* state causes the release of the free energy for transition from *T* to *R*.

The theory presented here, which is based on the allosteric model, is rather incomplete. It has not been shown that stimulations by disaccharides can be interpreted by the same model. It may be possible, however, to describe them with the allosteric model by using the constants in equation (D7), based on an assumption that one molecule of disaccharides can combine with each subunit at two different parts of the monosaccharide. We have assumed in this model (in the classical complex model, also) that there are only identical receptor sites, $S_G S_F$, but there could be other types of receptor sites, for example, $S_G S_G$ and $S_F S_F$. Such an additional assumption might give better agreement with the results of experiments on fructose effects on glucose stimulation, though a discrepancy has been pointed out between the theoretical curve and experimental values at low concentrations of fructose in Fig. 18 (curve C).

Monod et al. (1965) have based their theory on polymers of identical subunits, in which case they have proved that any intermediate state such as RT is unstable and can be neglected. Some recent papers (Antonini, Bucci, Fronticelli, Wyman, and Rossi-Fanelli, 1965; Tyuma, Benesch, and Benesch, 1966) show, however, that artificially synthesized hemoglobin molecules composed of the same four subunits have weaker allosteric activities compared with those of two α and two β chains. Therefore, it may be justifiable to assume that the two subunits introduced here have different structures.

At present, we have insufficient data to decide whether the classical complex model or the allosteric one is really correct, though the latter seems to give a somewhat better interpretation of the experimental results.

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